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First tardigrade records from San Pedro Mártir, Baja California, Mexico

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Abstract

A total of 66 specimens representing four tardigrade taxa were found in 14 moss and lichen samples collected from the bark of Jeffrey's Pine, *Pinus jeffreyi* Balf., in the Sierra de San Pedro Mártir (Baja California, north-western Mexico). Two taxa were identified to the species level (*Echiniscus becki* Schuster & Grigarick, 1966 and *E. blumi* Richters, 1903), and two to the genus level (*Milnesium* sp. and *Ramazzottius* sp.). These are the first tardigrade records from the state of Baja California, and they increase the known tardigrade diversity of Mexico to 84 species.

Keywords

Biodiversity, distribution, Tardigrada, *Echiniscus blumi*, *Echiniscus becki*

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Introduction

The Sierra de San Pedro Mártir (SSPM) is the highest mountainous system that runs through the entire peninsula of Baja California, Mexico (Fig. 1). The SSPM has an area of approximately 73,000 ha and a maximum elevation of 3100 m a.s.l. The vegetation of SSPM is mainly represented by coniferous forest, including *Abies concolor* (Gord. & Glend.) Lindl. ex Hildebr., *Pinus cuadrifolia* Parl. ex Sudw., *P. monophyla* Torr. & Frém., *P. contorta* ssp. *murrayana* (Grev. & Balf.) Critchf., and *P. jeffreyi* Balf., the dominant species (Delgadillo 2004).

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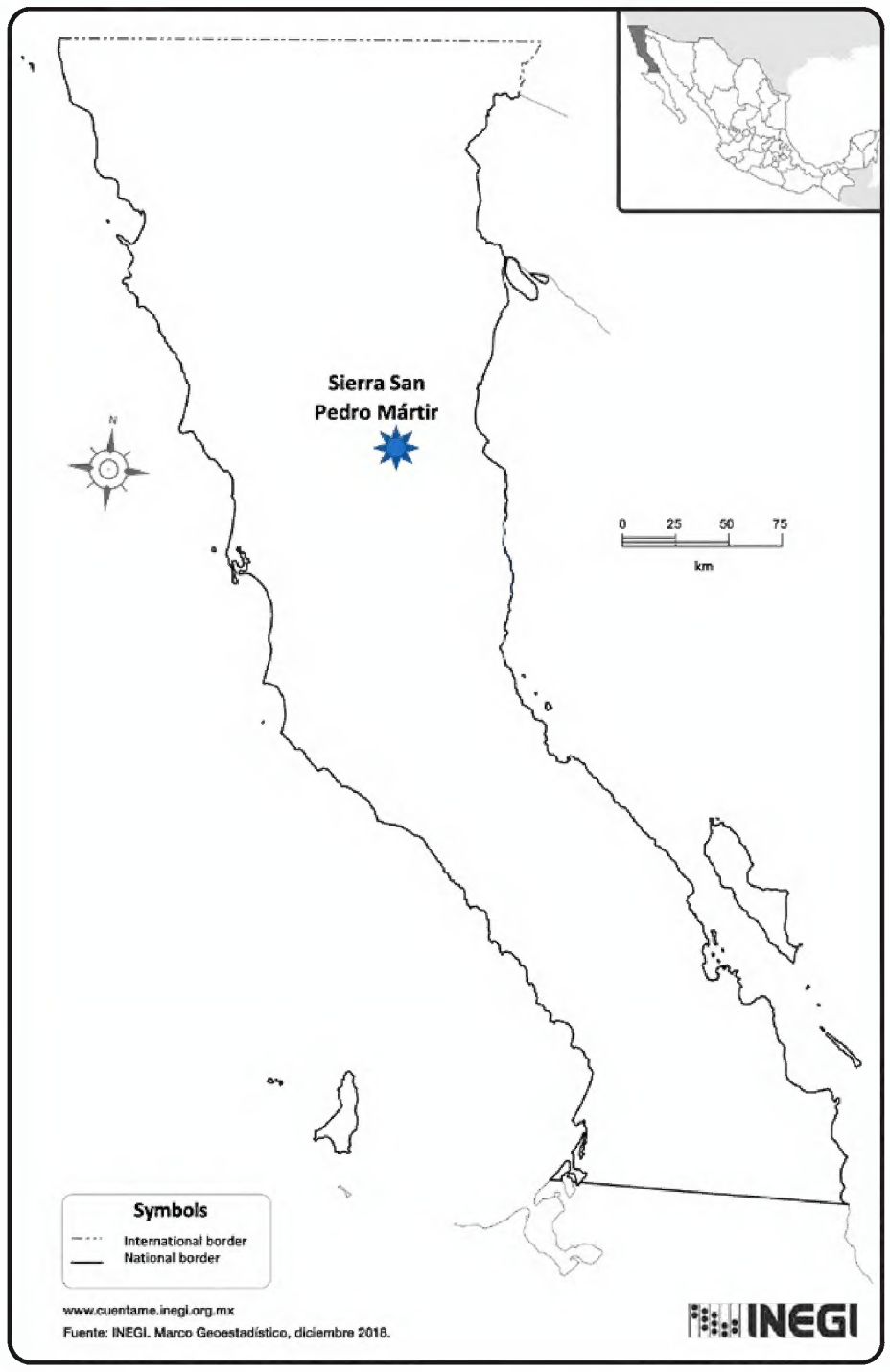

Figure 1. Location of the collection site within the Sierra de San Pedro Mártir, Baja California, Mexico. Map source: Instituto de Estadística, Geografia e Informática, https://www.inegi.mx).

Although the highest peaks of the SSPM are known to have low humidity and are ideal for astronomical observations (Carrasco et al. 2017), the proximity to the Pacific Ocean on the west and the Gulf of California on the east, as well as its environmental conditions, contribute to the formation of fog, mainly on the mountain slopes, which favours large plant formations. Thus, lichens associated with the *Pinus jeffreyi* forests form favourable microenvironments for the tardigrade fauna.

There are over 1,300 described species of tardigrades worldwide (Guidetti and Bertolani 2005; Degma and Guidetti 2007; Degma et al. 2020). Currently, the number of tardigrade species in Mexico is relatively small, numbering 82 species (64 limnoterrestrial, two freshwater, and 16 marines: May 1948; Schuster 1971; Beasley 1972; Pilato and Lisi 2006; Kaczmarek et al. 2011; Kaczmarek et al. 2014; Léon-Espinosa et al. 2019; Moreno-Talamantes et al. 2019, 2020; Moreno-Talamantes and Léon-Espinosa 2019; Pérez-Pech et al. 2020; Dueñas-Cedillo et al. 2020). These low numbers are most likely a result of a few tardigrade studies carried out in the country. The northwest region of Mexico is among the least studied, with single records from the states of Sonora (endemic species *Haplomacrobiotus hermosillensis* May, 1948), and Sinaloa (*Cornechiniscus lobatus* Ramazzotti, 1943) (Beasley 1972), and there are no records whatsoever from the state of Baja California. Here, we present a study of tardigrades from the north-western Mexico, reporting the first findings from the state of Baja California.

Methods

We collected 39 moss and 19 lichen samples (*Pyxine cocoes* (Sw.) Nyl. and *Usnea* sp.) from the bark of *Pinus jeffreyi* during the exploration to the Sierra de San Pedro Mártir in Ensenada, Baja California, Mexico. Our samples were all collected in the surroundings of the National Astronomical Observatory in May 2018. Samples were preserved in paper bags and georeferenced using a GPS receiver. We used standard methods for processing our limnoterrestrial tardigrade samples (Stec et al. 2015), which were rehydrated with spring water for 6 h. Moss and lichen samples were squeezed, and the obtained material was observed under an Olympus SZ 110AL2X-2 stereomicroscope. The specimens found were fixed in permanent slides with polyvinyl alcohol with Elvanol and lactophenol (BioQuip Products Inc., catalogue number 6371A) following the protocol of Moreno-Talamantes et al. (2015) and Morek et al. (2016). The specimens were examined under an AmScope T690C-PCT200-PL contrast phase microscope and an Olympus CX31 microscope with AmScope MU1000 digital camera.

Tardigrade species were identified using the taxonomic keys by Ramazzotti and Maucci (1983) and Kristensen (1987), as well as using the original descriptions and redescriptions of relevant species (Schuster and Grigarick 1966; Michalczyk et al. 2012; Stec et al. 2018) and the work of Gąsiorek et al. (2019). Morphological and taxonomical terminology for heterotardigrades follows Kristensen (1987) and further modifications introduced by Gąsiorek et al. (2017, 2019), Gąsiorek and Michalczyk (2020), and Morek et al. (2020). Claw configuration in *Milnesium* is given following Michalczyk et al. (2012). Specimens were deposited in the Colección Carcinológica-FCB-UANL in the Department of Entomology and Arthropods, Facultad de Ciencias Biológicas of the Universidad Autónoma de Nuevo León (Mexico).

Results

In total, 66 tardigrade specimens representing four taxa were found in six moss and eight lichen samples.

Phylum Tardigrada Doyère, 1840
Class Heterotardigrada Marcus, 1927
Order Echiniscoidea Richters, 1926
Family Echiniscidae Thulin, 1928
Genus *Echiniscus* C.A.S. Schultze, 1840

***Echiniscus blumi* Richters, 1903**

New records (Fig. 2). MEXICO – **Baja California** • Ensenada, Sierra San Pedro Mártir; 31°02′15″N, 115°27′06″W; 3000 m a.s.l.; 3.V.2018; P.G. Nuñez leg.; 8 adults;

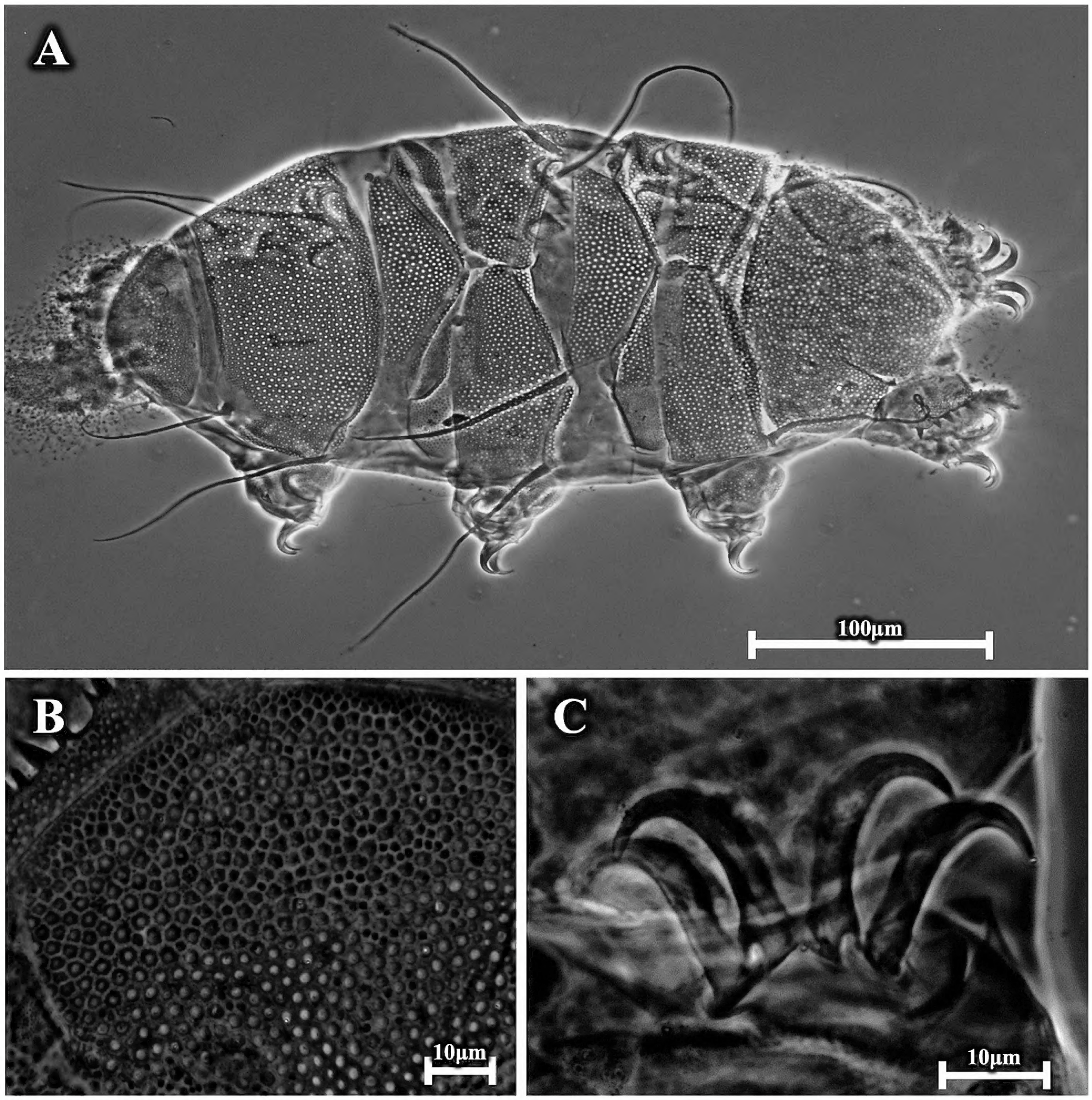

Figure 2. *Echiniscus blumi*. **A.** Habitus in dorsal view. **B.** Details of dorsal cuticle showing polygonal dots. **C.** Claw II. Scale bars: A = 100 µm; B, C = 10 µm.

on *Pyxinie cocoes* in *Pinus jeffreyi* • Ensenada, Sierra San Pedro Mártir; 31°02′45″N, 115°28′06″W; 3000 m a.s.l.; 3.V.2018; G.A. León -Espinosa leg.; 4 adults; in *Usnea* sp. FCB-UANL TARDI-12/15-0126-127.

Identification. The collected specimens correspond perfectly with the descriptions by Ramazzotti and Maucci (1983) and redescription of the *Echiniscus blumi–canadensis* series by Guil (2008). Body appendage configuration: *A-B-C-C*^{d}*-D-*(*D*^{d}); all in the form of cirri, except for *D*^{d} which is a spine (absent in some specimens).

Remarks. The new records represent the first documentation of this species in Mexico.

Echiniscus becki Schuster & Grigarick, 1966

New record (Fig. 3). MEXICO – **Baja California** • Ensenada, Sierra San Pedro Mártir; 31°02′15″N, 115°27′06″W; 3000 m a.s.l.; 4.V.2018; G.A. León-Espinosa leg.; 4 adults; in moss; TARDI-12/15-0157-0160.

Identification. The specimens correspond perfectly to the original description (Schuster and Grigarick 1966). Body appendage configuration: *A-C-D* (all cirri); only internal claws IV have spurs. Sculpture of dorsal plates with polygons.

Remarks. This species has been to date only from California, USA. This Mexican record expands the known geographic range of the species to the south.

Class Eutardigrada Richters, 1926
Order Apochela Schuster, Nelson, Grigarick & Christenberry, 1980
Family Milnesiidae Ramazzotti, 1962
Genus *Milnesium* Doyère, 1840

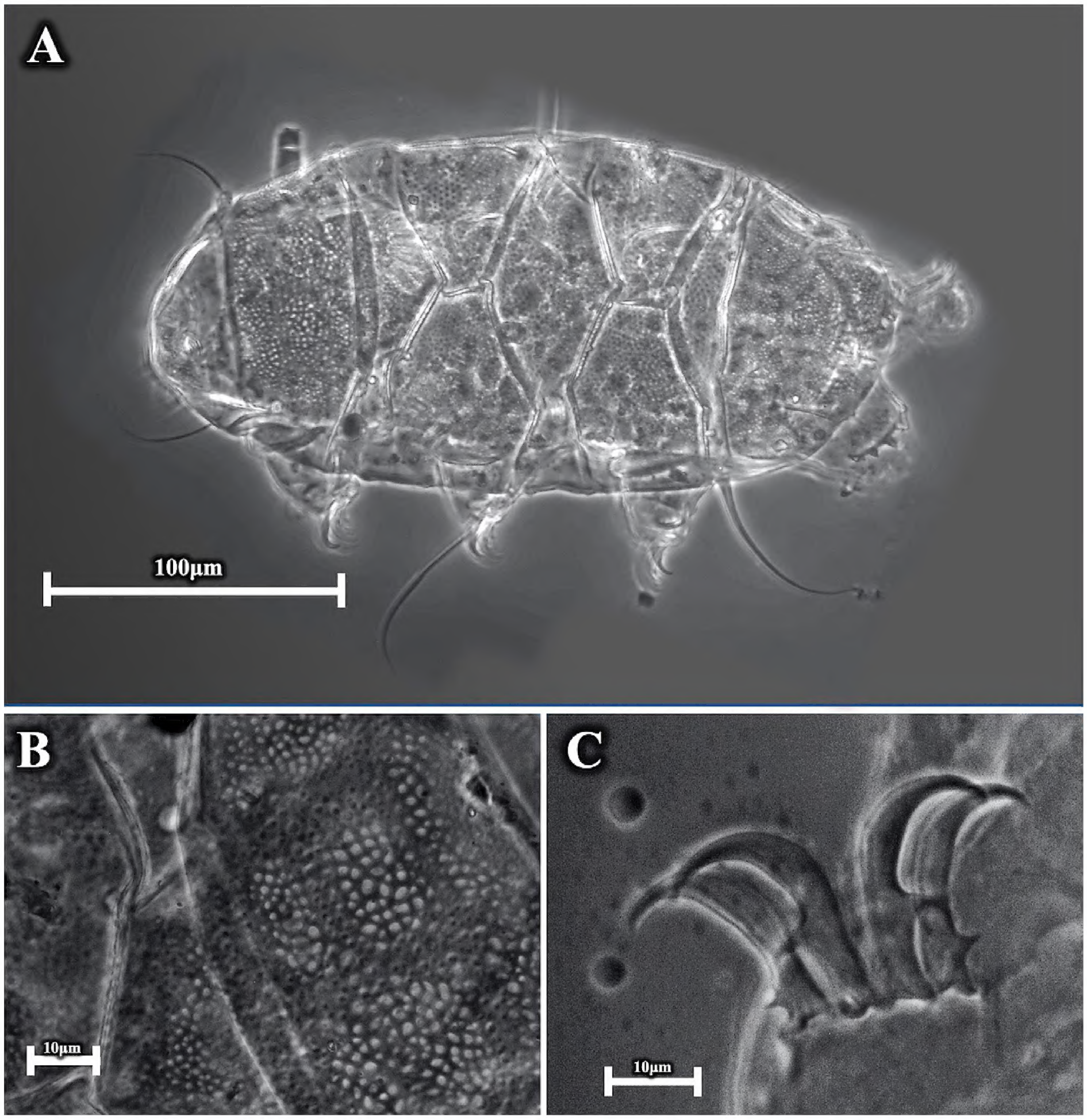

Figure 3. *Echiniscus becki*. **A.** Habitus in dorsal view. **B.** Details of dorsal cuticle showing polygonal dots. **C.** Claw IV. Scale bars: A = 100 µm; B = 20 µm.

***Milnesium* sp.**

New records. MEXICO – **Baja California** • Ensenada, Sierra San Pedro Mártir; 31°02′04″N, 115°28′08″W; 3.V.2018; G.A. León-Espinosa; 3 adults; moss • Ensenada, Sierra San Pedro Mártir; 31°02′15″N, 115°27′06″W; 3000 m a.s.l.; 3.V.2018; G.A. León-Espinosa; 13 adults; moss.

Identification. We found 16 adults in two moss samples. Although the specimens were found in different moss cushions, they exhibit a very similar morphology: the orange body colour, smooth cuticle, black eyes, and a [3-3]-[3-3] claw configuration. Given the few specimens that were collected, we cannot fully identify them to species. In a future integrative study, we will attempt to collect more specimens, study their complete ontogenetic development, and include DNA analyses to determine the species.

Order Parachela Schuster, Nelson, Grigarick & Christenberry, 1980

Superfamily Hypsibioidea Pilato, 1969 in Marley et al., 2011

Family Ramazzottiidae Sands, McInnes, Marley, Goodall-Copestake, Convey & Linse, 2008

Genus *Ramazzottius* Binda & Pilato, 1986

***Ramazzottius* sp.**

New records. MEXICO – **Baja California** • Ensenada, Sierra San Pedro Mártir; 31°02′4″N, 115°28′08″W; 3000 m a.s.l.; 3.V.2018; G.A. León-Espinosa; 23 adults; moss • Ensenada, Sierra San Pedro Mártir; 31°21′15″N, 115° 27′60″W; 4.V.2018; G.A. León-Espinosa; 11 adults; moss.

Identification. We found a mix of specimens exhibiting

smooth (7 specimens) and sculptured (17 specimens) dorsal cuticles in both moss samples. In all specimens, the dorsal cuticle was divided into five red-brown longitudinal and nine transverse stripes throughout the body. There is one embryonated egg that exhibits the *Ramazzottius* morphotype, but we are not certain to what species it may belong.

Remarks. We found that some specimens with a different cuticle have a different size (around 12% larger), but we do not know whether they are juvenile or belong to other species. In the absence of DNA data, we cannot determine if they represent a single species or multiple species.

Discussion

In this study we report two species new to Mexico, *Echiniscus blumi* and *Echiniscus becki*, which increase the number of recorded tardigrade species in Mexico from 82 to 84. Moreover, these two heterotardigrade species, together with further two partially identified eutardigrade taxa, constitute the first tardigrade records for the Sierra de San Pedro Mártir and the state of Baja California.

Echiniscus blumi was originally described from the Arctic but is considered cosmopolitan (McInnes 1994). However, as the type locality of this species is in the Arctic archipelago of Svalbard, it is likely to represent a species complex, tropical and subtropical records of *E. blumi* should be re-examined (Kaczmarek et al. 2015). Although our specimens morphologically fit the description of the species, they should be verified genetically against DNA sequences from Svalbard.

Our record of *E. becki* from Baja California means that the species can no longer be considered endemic to California, USA, but instead to a wider region at least including the mountains of northern Baja California. More study is necessary to determine the geographic limits of this species.

Given the relatively limited number of surveys and amount of territory in Mexico surveyed more studies on tardigrades are needed. Preferably, tardigrade studies should be carried out under the integrative taxonomy framework, as not only does it increase the taxonomic resolution of faunistic assays but also allows for the genetic verification of species records and phylogenetic analyses.

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Authors' Contributions

Conceptualization: PGN. Investigation: PGN, GALE, MEPS. Project administration: PGN. Visualization: PGN, GALE. Writing – review and editing: PGN, RV, AMT. Writing – original draft: GALE. Funding acquisition: RV. Supervision: GARA, AMT.

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